

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A method for typing of HLA class I alleles comprising the following steps of: ~~from (a) to (d);~~

(a) ~~A step, using~~ providing nucleotide sequence(s) encoding HLA class I alleles or a fragment thereof as ~~gene or nucleic acids containing their fragment~~ for a template for PCR;₇

(b) ~~(1) To non-selectively amplifying~~ amplify all HLA-A alleles, all HLA-B alleles, or all HLA-C alleles by a PCR method using a primer pair which can amplify all the HLA-A alleles, all the HLA-B alleles, or all the HLA-C alleles, or ~~(2) To selectively amplifying~~ amplify a specific group consisting of specific HLA-A alleles or a specific group of specific HLA-B alleles by a PCR method using a primer pair which is specific to the a common nucleotide sequence of ~~to alleles of the specific group consisting of the specific HLA-A alleles or the specific HLA-B alleles;~~₁

(c) ~~(b) A step to add~~ adding the resulting PCR products ~~above products amplified by the PCR method~~ to wells of microtiter plates, wherein each well is modified with a carboxyl group to covalently immobilize amino-modified DNA probes which can specifically hybridize with the sequence of at least one specific HLA-A allele, at least one specific HLA-B allele or at least one specific HLA-C allele, ~~and to hybridize;~~₁

(d) hybridizing the amplified products with the immobilized DNA probes, wherein the DNA probes are selected depending on the above amplified specific HLA class I gene or group;

(e) detecting hybridization of ~~(e) A step to detect as signals whether or not~~ the amplified products ~~are hybridized~~ with the immobilized DNA probes to produce a signal pattern; and

(f) determining ~~(d) A step to determine~~ the type of the HLA class I allele based on the signal pattern detected at the step (e) ~~(e)~~ according to the Typing Table.

2. (Currently Amended) The method ~~for typing of the HLA class I alleles claimed in~~ according to claim 1, wherein at least one primer of the primer pair is labeled.

3. (Currently Amended) The method ~~for typing of the HLA class I alleles claimed in~~ according to claim 2, ~~which comprises hybridizing~~ wherein hybridization of the amplified products ~~obtained by the PCR method~~ with the immobilized DNA probes, is determined by the steps of:

(i) adding an enzyme-conjugate which specifically bonds to the label of the amplified products thereto at the same time as or after the hybridization, and

(ii) adding a chromogenic substrate, a luminescent substrate or a fluorescent substrate to the mixture,

so as to detect as signals whether or not the amplified products are hybridized with the immobilized DNA probes.

4. (Currently Amended) The method ~~for typing of the HLA class I alleles claimed in~~ according to claim 3, wherein at least one primer of the primer pair is biotinylated and the enzyme-conjugate ~~which specifically bonds to the label of the amplified products obtained by the PCR method~~ is an enzyme-conjugated streptavidin.

5. (Currently Amended) The method ~~for typing of the HLA class I alleles claimed in~~ according to any one of claims 1 to 4, wherein the hybridization ~~of the amplified products obtained by the PCR method with the immobilized DNA probes~~ is performed in the presence of a solution containing formamide.

6. (Currently Amended) The method ~~for typing of the HLA class I alleles claimed in~~ according to claim 5, wherein hybridization occurs at a ~~the~~ reaction temperature for ~~hybridization of the amplified products obtained by the PCR method with immobilized DNA probes~~ is of about 37°C.

7. (Currently Amended) The method ~~for typing of the HLA class I alleles claimed in~~ according to claim 5, wherein the temperature for washing after hybridization of the amplified

products by the PCR method with the immobilized DNA probes and/or after the binding reaction of the label of the amplified products with the enzyme-conjugate is room temperature.

8. (Previously Presented) The method for typing of the HLA class I alleles claimed in claim 1, wherein the amino-modified DNA probe which can specifically hybridize with at least one specific HLA-A allele, at least one specific HLA-B allele or at least one specific HLA-C allele, is selected from the group consisting of A98T (SEQ ID No.:1), A98A (SEQ ID No.:2), A160A (SEQ ID No.:3), A239A (SEQ ID No.:4), A238A (SEQ ID No.:5), A240T (SEQ ID No.:6), A257TC (SEQ ID No.:7), A259AC (SEQ ID No.:8), A270T (SEQ ID No.:9), A282C (SEQ ID No.:10), A290T (SEQ ID No.:11), A299T (SEQ ID No.:12), A302G (SEQ ID No.:13), A355G (SEQ ID No.:14), A362TA (SEQ ID No.:15), A362TT (SEQ ID No.:16), A368A (SEQ ID No.:17), A368G (SEQ ID No.:18), A368T (SEQ ID No.:19), A402G (SEQ ID No.:20), A423T (SEQ ID No.:21), A448C (SEQ ID No.:22), A485A (SEQ ID No.:23), A524G (SEQ ID No.:24), A526T (SEQ ID No.:25), A527A (SEQ ID No.:26), A538CG (SEQ ID No.:27), A539A (SEQ ID No.:28), A539T (SEQ ID No.:29), A555T (SEQ ID No.:30), A559G (SEQ ID No.:31), A570CG (SEQ ID No.:32), A570GT (SEQ ID No.:33), A779A (SEQ ID No.:34), A843A (SEQ ID No.:35), BL1 (SEQ ID No.:36), BL3 (SEQ ID No.:37), BL4 (SEQ ID No.:38), BL5 (SEQ ID No.:39), BL9 (SEQ ID No.:40), BL10 (SEQ ID No.:41), BL11 (SEQ ID No.:42), BL24 (SEQ ID No.:43), BL25 (SEQ ID No.:44), BL34 (SEQ ID

No.:45), BL35 (SEQ ID No.:46), BL36 (SEQ ID No.:47), BL37 (SEQ ID No.:48), BL38 (SEQ ID No.:49), BL39 (SEQ ID No.:50), BL40 (SEQ ID No.:51), BL41 (SEQ ID No.:52), BL42 (SEQ ID No.:53), BL56 (SEQ ID No.:54), BL57 (SEQ ID No.:55), BL78 (SEQ ID No.:56), BL79 (SEQ ID No.:57), BL222A (SEQ ID No.:58), BL272GA (SEQ ID No.:59), BL226G (SEQ ID No.:60), BL292G (SEQ ID No.:61), BL292T (SEQ ID No.:62), BL361G (SEQ ID No.:63), BL409T (SEQ ID No.:64), BL512T (SEQ ID No.:65), BL538CG (SEQ ID No.:66), BL538G (SEQ ID No.:67), CC (SEQ ID No.:68), A-12 (SEQ ID No.:69), A-2 (SEQ ID No.:70), A-3 (SEQ ID No.:71), A-4 (SEQ ID No.:72), A-54 (SEQ ID No.:73), B-1 (SEQ ID No.:74), B-2 (SEQ ID No.:75), C-12 (SEQ ID No.:76), C-24 (SEQ ID No.:77), C-33 (SEQ ID No.:78), C-43 (SEQ ID No.:79), 134-g (SEQ ID No.:80), 134-A2 (SEQ ID No.:81), 353TCA1 (SEQ ID No.:82), 343A (SEQ ID No.:83), A34 (SEQ ID No.:100), A282CT (SEQ ID No.:101), A290TR (SEQ ID No.:102), A302GR (SEQ ID No.:103), A414A (SEQ ID No.:104), A468T (SEQ ID No.:105), A489A (SEQ ID No.:106), A502C (SEQ ID No.:107), A538TG (SEQ ID No.:108), BL39R (SEQ ID No.:109), BL50 (SEQ ID No.:110), BL77 (SEQ ID No.:111), BL272A (SEQ ID No.:112), BL263T (SEQ ID No.:113), BL527A (SEQ ID No.:114), BL570GT (SEQ ID No.:115), RA-2 (SEQ ID No.:116), RA-41 (SEQ ID No.:117), RB-28 (SEQ ID No.:118), 201g1 (SEQ ID No.:119), C206gR (SEQ ID No.:120), R341A (SEQ ID No.:121), R343g3 (SEQ ID No.:122), 353TCC (SEQ ID No.:123), 361T1 (SEQ ID No.:124), 361T368g (SEQ ID No.:125), 361T368T1 (SEQ ID No.:126), 369C (SEQ ID No.:127), 387g1 (SEQ ID No.:128), 526AC2 (SEQ ID No.:129),

538gAC (SEQ ID No.:130), complementary strands thereof and nucleic acids which comprises one to several bases are deleted from or added to the end of them.

9. Canceled.

10. (Withdrawn) A DNA probe used for a typing method of the HLA class I alleles, which is selected from the group consisting of A98T (SEQ ID No.:1), A98A (SEQ ID No.:2), A160A (SEQ ID No.:3), A239A (SEQ ID No.:4), A238A (SEQ ID No.:5), A240T (SEQ ID No.:6), A257TC (SEQ ID No.:7), A259AC (SEQ ID No.:8), A270T (SEQ ID No.:9), A282C (SEQ ID No.:10), A290T (SEQ ID No.:11), A299T (SEQ ID No.:12), A302G (SEQ ID No.:13), A355G (SEQ ID No.:14), A362TA (SEQ ID No.:15), A362TT (SEQ ID No.:16), A368A (SEQ ID No.:17), A368G (SEQ ID No.:18), A368T (SEQ ID No.:19), A402G (SEQ ID No.:20), A423T (SEQ ID No.:21), A448C (SEQ ID No.:22), A485A (SEQ ID No.:23), A524G (SEQ ID No.:24), A526T (SEQ ID No.:25), A527A (SEQ ID No.:26), A538CG (SEQ ID No.:27), A539A (SEQ ID No.:28), A539T (SEQ ID No.:29), A555T (SEQ ID No.:30), A559G (SEQ ID No.:31), A570CG (SEQ ID No.:32), A570GT (SEQ ID No.:33), A779A (SEQ ID No.:34), A843A (SEQ ID No.:35), BL1 (SEQ ID No.:36), BL3 (SEQ ID No.:37), BL4 (SEQ ID No.:38), BL5 (SEQ ID No.:39), BL9 (SEQ ID No.:40), BL10 (SEQ ID No.:41), BL11 (SEQ ID No.:42), BL24 (SEQ ID No.:43), BL25 (SEQ ID No.:44), BL34 (SEQ ID No.:45), BL35 (SEQ ID No.:46), BL36 (SEQ ID No.:47), BL37 (SEQ ID No.:48), BL38

(SEQ ID No.:49), BL39 (SEQ ID No.:50), BL40 (SEQ ID No.:51), BL41 (SEQ ID No.:52), BL42 (SEQ ID No.:53), BL56 (SEQ ID No.:54), BL57 (SEQ ID No.:55), BL78 (SEQ ID No.:56), BL79 (SEQ ID No.:57), BL222A (SEQ ID No.:58), BL272GA (SEQ ID No.:59), BL226G (SEQ ID No.:60), BL292G (SEQ ID No.:61), BL292T (SEQ ID No.:62), BL361G (SEQ ID No.:63), BL409T (SEQ ID No.:64), BL512T (SEQ ID No.:65), BL538CG (SEQ ID No.:66), BL538G (SEQ ID No.:67), CC (SEQ ID No.:68), A-12 (SEQ ID No.:69), A-2 (SEQ ID No.:70), A-3 (SEQ ID No.:71), A-4 (SEQ ID No.:72), A-54 (SEQ ID No.:73), B-1 (SEQ ID No.:74), B-2 (SEQ ID No.:75), C-12 (SEQ ID No.:76), C-24 (SEQ ID No.:77), C-33 (SEQ ID No.:78), C-43 (SEQ ID No.:79), 134-g (SEQ ID No.:80), 134-A2 (SEQ ID No.:81), 353TCA1 (SEQ ID No.:82), 343A (SEQ ID No.:83), A34 (SEQ ID No.:100), A282CT (SEQ ID No.:101), A290TR (SEQ ID No.:102), A302GR (SEQ ID No.:103), A414A (SEQ ID No.:104), A468T (SEQ ID No.:105), A489A (SEQ ID No.:106), A502C (SEQ ID No.:107), A538TG (SEQ ID No.:108), BL39R (SEQ ID No.:109), BL50 (SEQ ID No.:110), BL77 (SEQ ID No.:111), BL272A (SEQ ID No.:112), BL263T (SEQ ID No.:113), BL527A (SEQ ID No.:114), BL570GT (SEQ ID No.:115), RA-2 (SEQ ID No.:116), RA-41 (SEQ ID No.:117), RB-28 (SEQ ID No.:118), 201g1 (SEQ ID No.:119), C206gR (SEQ ID No.:120), R341A (SEQ ID No.:121), R343g3 (SEQ ID No.:122), 353TCC (SEQ ID No.:123), 361T1 (SEQ ID No.:124), 361T368g (SEQ ID No.:125), 361T368T1 (SEQ ID No.:126), 369C (SEQ ID No.:127), 387g1 (SEQ ID No.:128), 526AC2 (SEQ ID No.:129), 538gAC (SEQ ID No.:130), complementary strands thereof and

nucleic acids which comprises one to several bases are deleted from or added to the end of them.

11. (Withdrawn) A primer used for a typing method of the HLA class I alleles, which is selected from the group consisting of BASF-1 (SEQ ID No.:88), BASR-1 (SEQ ID No.:89), CGA011 (SEQ ID No.:90), CGA012 (SEQ ID No.:91), AIn3-66C (SEQ ID No.:92), 5BCIn37-34C (SEQ ID No.:96), 5BCIn37-24g (SEQ ID No.:97) and BCIn37-34g2 (SEQ ID No.:99).

12. (Withdrawn) A kit for typing of the HLA class I alleles, which is used for the method claimed in claim 1.

13. (Withdrawn) A reagent for typing of the HLA class I alleles, which is used for the method claimed in claim 1.

14. (Withdrawn) A kit for typing of the HLA class I alleles, which comprises the DNA probe claimed in claim 10.

15. (Withdrawn) A reagent for typing of the HLA class I alleles, which comprises the probe claimed in claim 10.

16. (Withdrawn) A kit for typing of the HLA class I alleles, which comprises the primer claimed in claim 11.

17. (Withdrawn) A reagent for typing of the HLA class I alleles, which comprises the primer claimed in claim 11.

18. (Withdrawn) A method for detecting a specific base sequence, wherein hybridization is performed in a hybridization buffer containing 10% to 25% formamide, at 32°C to 42°C, using a probe of 14 to 24 or more of bases.

19. (Withdrawn) The method claimed in claim 18, wherein the hybridization buffer contains 0.25M di-sodium hydrogenphosphate, 7% sodium dodecyl sulfate, 1% bovine serum albumin, 0.03M phosphoric acid, 0.5M ethylenediaminetetraacetic acid and 10% to 25% formamide.

20. (Withdrawn) The method claimed in claim 18 or 19, wherein the temperature for washing after the hybridization is room temperature.

21. (Withdrawn) The method claimed in claim 18, wherein the probes are hybridized with amplified products by the PCR method.

22. (Withdrawn) The method claimed in claim 21, wherein at least one of the primer pair is labeled.

23. (Withdrawn) The method claimed in claim 18, wherein nucleic acids are hybridized with the probes immobilized on a support.

24. (Withdrawn) The method claimed in claim 21, which comprises hybridizing the amplified products obtained by the PCR method with the immobilized DNA probes, adding an enzyme-conjugate which specifically bonds to a label of the amplified products thereto at the same time or after the hybridization, and adding a chromogenic substrate, a luminescent substrate or a fluorescent substrate to the mixture, to detect as signals whether or not the amplified products are hybridized with the immobilized DNA probes.

25. (Withdrawn) The method claimed in claim 24, wherein the label is a biotin and the enzyme-conjugate is an enzyme-conjugated streptavidin.

26. (New) A method for typing HLA class I alleles comprising the steps of:

(a) providing nucleotide sequence(s) encoding HLA class I alleles or a fragment thereof as a template for PCR;

(b) non-selectively amplifying all HLA-A alleles, all HLA-B alleles, or all HLA-C alleles by PCR using a primer pair which can amplify all the HLA-A alleles, all the HLA-B alleles, or all the HLA-C alleles, or selectively amplifying a specific group of HLA-A alleles or a specific group of HLA-B alleles by PCR using a primer pair which is specific to a common nucleotide sequence of the specific group;

(c) adding the resulting PCR products to wells of microtiter plates, which are immobilized DNA probes which can specifically hybridize with the sequence of at least one specific HLA-A allele, at least one specific HLA-B allele or at least one specific HLA-C allele;

(d) hybridizing the amplified products with the immobilized DNA probes, wherein the DNA probes are selected depending on the above amplified specific HLA class I gene or group;

(e) detecting hybridization of the amplified products with the immobilized DNA probes to produce a signal pattern; and

(f) determining the type of the HLA class I allele based on the signal pattern detected at the step (e) according to the Typing Table.

27. (New) The method according to any one of claims 1 to 4, wherein hybridization occurs at a reaction temperature of about 37°C.

28. (New) The method according to any one of claims 1 to 4, wherein the temperature for washing after hybridization of the amplified products by the PCR method with the immobilized DNA probes and/or after the binding reaction of the label of the amplified products with the enzyme-conjugate is room temperature.